# 8-Hydroxy-2′-deoxyguanosine, A Major Mutagenic Oxidative DNA Lesion, and DNA Strand Breaks in Nasal Respiratory Epithelium of Children Exposed to Urban Pollution

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Southwest metropolitan Mexico City children are repeatedly exposed to high levels of a complex mixture of air pollutants, including ozone, particulate matter, aldehydes, metals, and nitrogen oxides. We explored nasal cell 8-hydroxy-2′-deoxyguanosine (8-OHdG), a major mutagenic lesion producing G→T transversion mutations, using an immunohistochemical method, and DNA single strand breaks (ssb) using the single cell gel electrophoresis assay as biomarkers of oxidant exposure. Nasal biopsies from the posterior inferior turbinate were examined in children in grades one through five, including 12 controls from a low-polluted coastal town and 87 Mexico City children. Each biopsy was divided for the 8-OHdG and DNA ssb assays. There was an age-dependent increase in the percentage of nasal cells with DNA tails > 10 μm in Mexico City children: 19 ± 9% for control cells, and 43 ± 4, 50 ± 16, 56 ± 17, 60 ± 17 and 73 ± 14%, respectively, for first through fifth graders ($p < 0.05$). Nasal ssb were significantly higher in fifth graders than in first graders ($p < 0.05$). Higher levels (2.3- to 3-fold) of specific nuclear staining for 8-OHdG were observed in exposed children as compared to controls ($p < 0.05$). These results suggest that DNA damage is present in nasal epithelial cells in Mexico City children. Persistent oxidative DNA damage may ultimately result in a selective growth of pr eneoplastic nasal initiated cells in this population and the potential for nasal neoplasms may increase with age. The combination of 8-OHdG and DNA ssb should be useful for monitoring oxidative damage in people exposed to polluted atmospheres. *Key words*: air pollution, biomarker, children, DNA damage, DNA strand breaks, 8-hydroxy-2′-deoxyguanosine, Mexico City, nasal epithelium, oxidative DNA damage, reactive nitrogen species, reactive oxygen species. *Environ Health Perspect* 107:469–474 (1999). [Online 4 May 1999]
*http://ehpnet1.niehs.nih.gov/docs/1999/107p469-474calderon-garciduenas/abstract.html*

Despite complex DNA repair mechanisms, oxidatively modified DNA is present in human tissues; the damaged nucleosides accumulate with age in both nuclear and mitochondrial DNA (*1–6*). The respiratory apparatus is exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS) from a variety of sources, including air pollutants, phagocytic cells, aerobic bacteria, and reactive xenobiotic-drug metabolites (*4,7–10*). Oxidants can injure cells through damage to DNA, thus producing structural alterations, affecting cytoplasmic and nuclear signal transduction pathways and modulating the activity of proteins and genes that respond to stress and which act to regulate the genes related to cell proliferation. All of these injuries can lead to cell death or malignant transformation (*7,8,10,11*). The contribution of oxidative stress to the development of human cancer will depend on several factors, including the extent of DNA damage, levels of antioxidant defenses, DNA repair systems, and the cytotoxic effects of ROS in large amounts (*8*).

Validation studies of biologic markers are necessary to follow populations repeatedly exposed to atmospheric pollutants, including ozone, particulate matter, aldehydes, and nitrogen oxides, with the ultimate goal of preventing cancer and other diseases (*12*). The significant interindividual variation in biomarkers apparently reflects the modulating effect of genetic and acquired susceptibility factors (*12*). Identification of subpopulations at higher risk of detrimental effects from air pollutant exposure is needed (*13*).

The nasal epithelium offers an accessible, easily monitored tissue. As the first site of contact of the respiratory tract with the environment, it is a common site for absorption of many gases and vapors and for particle deposition—a prime site for toxicant-induced pathology (*14,15*). Human nasal neoplasms may be related to exposure to environmental factors, including tobacco smoke, dust, metals, aldehydes, chemicals, and viruses (*16,17*). Oxidative DNA damage is generally regarded as a significant contributory cause of cancer for some environmental pollutants (*18*). In view of its potential importance, we compared two methods for measuring DNA damage—8-hydroxy-2′-deoxyguanosine (8-OHdG) and DNA strand breaks—in nasal epithelial cells from children chronically exposed to atmospheric air pollutants. This paper focuses on the usefulness of these two biomarkers in the particular setting of chronic exposure to high concentrations of air pollutants, and the potential relevance of these assay findings in the development of nasal neoplasms.

## Materials and Methods

***Chemicals.*** RNase, DNase, proteinase K, polyethylene glycol, EDTA, phosphate-buffered saline (PBS), and $H_2O_2$ were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 was obtained from ICN Pharmaceutical, Inc. (Costa Mesa, CA). Permount was purchased from Fisher Scientific (Pittsburgh, PA). Mouse ABC and 3,3′-dimethylaminoazobenzene kits were obtained from Vector Laboratories (Burlingame, CA).

***Pollutant methodology.*** Atmospheric pollutants were monitored at the Pedregal Station, located in Southwest Metropolitan Mexico City, Mexico (SWMMC), downwind of the major diurnal emissions in metropolitan Mexico City and 3 miles or less from the children's neighborhood. Concentration data for ozone ($O_3$), nitrogen dioxide ($NO_2$), nitrogen oxides ($NO_x$), sulfur dioxide ($SO_2$), and suspended particles < 10 μm ($PM_{10}$) were reviewed for the last 12 years (1986–1998) and pertinent data were included in this work. For $O_3$ exposures we examined three measures: the maximal concentrations, the number of hours equal to or above the U.S. National Ambient Air Quality Standards (NAAQS), and the time of occurrence of pollutant peaks. The data from Manzanillo, Mexico (the control site on the Pacific coast) were obtained from the Capitania del Puerto.

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Special thanks to A. Carnevale, (Instituto Nacional de Pediatria, Mexico City) for her constant support and encouragement. The authors are indebted to C. Lawler (University of North Carolina at Chapel Hill) for her statistical advice and to J. Villarreal-Calderón for the control population clinical assistance.

This study was supported in part by NIEHS training grant T32 ES07126, NIH ES05116, CA 73330, ES09089, and CD902908.

Received 5 June 1998; accepted 5 November 1998.

***Study population.*** The project was approved by the Instituto Nacional de Pediatria Review Boards for Human Studies (Mexico City, Mexico), and informed written consent was obtained from the children's parents. The study group consisted of 98 children, including a control population of 12 children and an exposed SWMMC group of 86 children. All participants in the study had a personal negative history of active smoking and environmental tobacco smoke exposure. The questionnaire information obtained from each child and parent included age; place and length of residency; average daily outdoor time; type of physical activity and time of the day when it typically occurs; parents' occupational history; family history of respiratory diseases; personal history of allergies and respiratory and otolaryngology symptoms (including epistaxis, rhinorrea, quality and quantity of mucus, nasal dryness, nasal obstruction, cough, and thoracic pain); and respiratory illness in the previous 3 months. Children with a history of ear–nose–throat surgical procedures, in need of treatment for atopic or infectious rhinitis, bronchitis, asthma, or allergic diseases, and those taking vitamin supplements or with known exposures to specific substances (solvents, paints, metals, photocopying machines) were excluded from the study. Children identified as ineligible included one child taking daily vitamins, three children with ethmoidal sinusitis and one child exposed to paints and solvents at home. The control children included five girls and seven boys, with an average age of 10.75 ± 1.05 years, with daylight outdoor exposure of 9.3 ± 1.2 hr. These children lived in a low-polluted Pacific port, they seldom left their town, and they had never been to a large city (i.e., > 50,000 inhabitants) including Mexico City. The exposed group included 33 girls and 53 boys ranging in age from 6 to 13 years, with an outdoor daily exposure of 7.2 ± 2.4 hr. These children were subdivided into five groups reflecting school grade level (first–fifth). SWMMC children were lifelong residents in the area, attended the same school, had the same socioeconomic level, and lived in the same neighborhood.

***Nasal biopsies.*** We obtained samples of nasal epithelium from the posterior inferior nasal turbinates with a disposable plastic curette (Rhino-Probe, ASI, Arlington, TX) under direct visual inspection. Occasional tearing of the ipsilateral eye and sneezing were recorded after the procedure. The biopsy sample was immediately immersed in 1 mL cold RPMI 1640 medium and reserved for the propidium iodide (PI) exclusion dye assay viability (*19*), the single cell gel electrophoresis (SCGE) assay (*20*), and 8-OHdG immunohistochemistry (*21*). SWMMC nasal samples were easily desegregated into single cells by gently shaking the glass tube; control samples required mincing with a scalpel blade and vortexing for 10 sec. Two hundred microliters of the cell suspension was taken for the SCGE assay and 100 µL for the PI viability test. The remaining 700 µL was centrifuged at 1,000 rpm for 10 min. The cell pellet was resuspended in sucrose buffer (0.25 M sucrose, 1.8 mM Ca $Cl_2$, 25 mM KCl, and 50mM Trizma base, pH 7.5) to a final volume of 1 mL. One hundred microliters of cell suspension was added to 300 µL carbowax–ethanol buffer (0.8% polyethylene glycol in 70% ethanol). After briefly vortexing, the cell suspension was cytospun at 300 rpm for 5 min onto each of two slides precoated with 0.2% poly-D-lysine. Slides were air dried, fixed in 95% ethanol, and stored at -80°C until staining.

***Single gel cell electrophoresis assay.*** We assessed DNA damage by the SCGE assay (*20*) and analysis was carried out as described previously (*21*). Briefly, the single nasal cell suspension volume was adjusted to 50,000 cell/50 µL RPMI. The 50-µL cell suspension sample was mixed with 50 µL low-melting agarose at 37°C and placed on precleaned microscope slides (Fisher fully frosted slides, Fisher Scientific), which were already covered with a thin layer of 0.5% normal-melting agarose. The slides were kept at 4°C for 5 min to allow solidification of the agarose, then immersed in a freshly made cold 4°C lysing solution (2.5M NaCl, 100 mM $Na_2$, EDTA, 10 mM Tris, pH 10, and 1% Triton) for 1 hr to lyse the cells. The slides were then removed from the lysing solution and placed on a horizontal gel electrophoresis unit (Easy Cast, Model B2, Owl Scientific, Inc., Woodburn, MA). The unit was filled with fresh electrophoretic alkaline buffer (1 mM EDTA and 300 mM NaOH), and the slides were allowed to sit for 20 min to permit unwinding of DNA before electrophoresis. Samples were electrophoresed for 20 min at 25 V and 300 mA. After electrophoresis, the slides were washed gently with 0.4 M Tris, pH 7.5, then stained with 25 µL of 20 µg/mL ethidium bromide in distilled water. Observations were made using an Olympus AH-2 microscope (Alta Technología Laboratorios, Tokyo, Japan) equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. We analyzed a minimum of 50 randomly selected cells per sample and the score was based on the observations of one slide reader, thus minimizing variability due to subjective scoring. To quantitate DNA migration, we measured with an ocular micrometer the DNA pattern length of individual cells in two replicate slides from the same nasal sample. For each cell, the length of the image (nucleus plus migrated DNA) was measured in micrometers at a 250-fold magnification. To simplify data analysis, the distribution of DNA migration was arbitrarily divided into five groups: < 10 µm, 10–40 µm, 41–80 µm, 81–120 µm, and > 121 µm. DNA from most control cells (peripheral blood fresh lymphocytes) appeared as a round pattern that did not migrate in the gel. The SCGE assay utilizes the principle that levels of single strand breaks (ssb) are proportional to the DNA migration distance in an electrophoresis field under the conditions used.

***Immunohistochemical staining for 8-OHdG.*** Immunohistochemical staining for 8-OHdG was carried out as described previously, using monoclonal antibody 1F7 (*22*). Briefly, slides were washed with 1 × PBS twice, then treated with RNase (100 µg/mL) in Tris buffer (pH 7.5; 10 mM Trizma base, 1 mM EDTA, and 0.4 M NaCl) at 37°C for 1 hr. After washing with PBS, we treated the cells with proteinase K (10 µg/mL) at room temperature for 7 min. After rinsing with PBS, the DNA was denatured by treatment with 4 N HCl for 7 min at room temperature.The pH was adjusted with 50 mM Trizma base for 5 min at room temperature. After washing with PBS we treated the cells with 10% normal horse serum in 10 mM Tris (pH 7.5) at 37°C for 1 hr to block nonspecific binding sites, then incubated with primary antibody 1F7 (1:30 dilution of hybridoma supernatant) at 4°C overnight. After washing with PBS, we treated the cells with goat–antimouse IgG conjugated to biotin at 37°C for 30 min. Endogenous peroxidase was blocked by treating the cells with 3% $H_2O_2$ in methanol for 30 min at room temperature. After washing with PBS, ABC reagent (avidin and biotinylated horseradish peroxidase complex) was added, and the slides were incubated for 30 min at 37°C. Incubation was followed by PBS and 1% Triton X-100 in PBS washes. To localize peroxidase, cells were treated with diaminobenzidine for 7 min at room temperature. Finally, slides were washed with water, dehydrated by a series of 95 and 100% ethanol and xylene washes, mounted with cover glasses using Permount and evaluated with a Cell Analysis System CAS 200 microscope (Becton-Dickinson, San Jose, CA). The relative intensity of nuclear staining of 50 randomly selected cells was measured using the Cell Measurement Program (Becton-Dickinson) software package. Data presented are the object average absorbance

multiplied by 1,000. To demonstrate specificity, cells were pretreated with DNase (100 µg/µL for 1 hr at 37°C) before staining, and stained with a nonspecific antiserum 8G1 (1:10 dilution), recognizing DNA damage produced by the photoactivated drug 8-methoxypsoralen, or with antibody 1F7 preabsorbed with 8-OHdG (1 µg/µL) for 20 min at room temperature before use.

***Statistical analysis.*** Analysis was performed using the Instat program (GraphPad, San Diego, CA). The following statistical procedures were used: first, one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test compared control versus exposed groups in the number of nasal cells with ssb and the quantitation of 8OHdG; second, Kruskal-Wallis nonparametric ANOVA test compared the differences in cell viabilities among the different groups. Data are presented as mean ± standard deviation and $p$-value of $< 0.05$ was used to determine statistical significance.

## Results

***Air quality data.*** SWMMC children are chronically exposed to a complex mixture of air pollutants, including high $O_3$ concentrations up to 0.48 ppm (*23*). The number of hours SWMMC children were exposed to $O_3 > 0.12$ ppm for the years from 1985 to 1996 are as follows: 40; 30; 740; 959; 1,124; 1,403; 1,561; 1,395; 1,146; 1,061; 1,249; and 1,080. SWMMC children were studied during the months of May and June 1996. The sampling dates and the previous-day ozone values follow:

- May 22, third and fourth graders (May 21 data: 6 hr > 0.08 ppm, max $O_3$ peak 0.157 ppm).
- May 27, first and fifth graders (May 26 data: 7 hr > 0.08 ppm, max $O_3$ peak 0.178 ppm).
- June 7, second graders (June 6 data: 5 hr > 0.08 ppm, max $O_3$ peak 0.225 ppm).

Children were specifically grouped in this way and, because we were particularly interested in the comparison of first and fifth graders, we collected their nasal samples the same day. In May and June 1996, there were 161 and 98 hr with $O_3 > 0.08$ ppm and maximal $O_3$ peaks of 0.232 and 0.261 ppm, respectively. $PM_{10}$ concentrations were, on average, 53 and 61 µg/m$^3$, respectively. $SO_2$ and $NO_2$ values were below their respective NAAQS. Formaldehyde and acetaldehyde concentrations ranged from 5.9 to 110 ppbv and 2 to 66.7 ppbv, respectively, with maximal peaks recorded between 800 and 1000 hours for acetaldehyde and between 1000 and 1200 hours for formaldehyde (*24,25*). Formaldehyde concentrations were higher on sunny days, coinciding with atmospheric stability and heavy smog conditions. Other pollutants reported in SWMMC air include: benzo[*a*]pyrene, benzo[*k*]fluoranthene, and volatile organic compounds (*26*). The control population was sampled in Manzanillo in May 1996. Averages for atmospheric and meteorological conditions for the season were 28°C, relative humidity 87%, $O_3 < 0.010$ ppm, and $PM_{10} < 14$ µg/m$^3$.

***Clinical data.*** Children in the control group reported no nasal or respiratory symptoms. The exposed children complained of nasal obstruction (63%), epistaxis (48%), nasal dryness (43%), and cough and chest discomfort (46%). Symptoms were more frequent in older children: nasal obstruction 46 versus 73%, epistaxis 30 versus 86%, nasal dryness 30 versus 80%, cough 46 versus 66%, and chest discomfort 25 versus 73% in first versus fifth graders, respectively.

***Detection of 8-OHdG.*** Representative biopsies indicating lighter nuclear staining from a control nasal sample as compared to a sample from an exposed child are shown in Figures 1A and 1B, respectively. As a control for the staining, cells from several positive subjects were restained after either treatment of the slide with DNase, or with primary antibody that had been preabsorbed with 8-OHdG before use. These treatments resulted in decreased nuclear staining and demonstrated the staining specificity. Relative staining intensity was quantitated and the mean nuclear staining for 8-OHdG in nasal cells from control children was 210 ± 122, whereas the exposed groups showed mean values of 627 ± 197, 577 ± 132, 657 ± 170, 647 ± 260, and 502 ± 218 for first through fifth graders, respectively (Figure 2). There was a significant difference in mean nuclear staining between control and exposed groups ($p < 0.05$), but no differences among the exposed groups themselves.

***SCGE assay.*** The percentage of nasal cells with DNA damage (ssb) in control children was 19 ± 9, and all migrated in the range of 10–40 µm. By contrast, the exposed children displayed an age-dependent increase in the percentage of cells with DNA tails > 10 µm: 43 ± 4, 50 ± 16, 56 ± 17, 60 ± 17, and 73 ± 14 for grades one through five, respectively ($p < 0.05$) (Figure 3). When we compared the DNA histogram distribution of ssb (> 10 to > 120 µm) between first and fifth graders, a shift to the right in the older children's histograms was noted; significantly more nasal cells with DNA tails in the ranges of 41–80 µm ($p < 0.05$) and 81–120 µm ($p < 0.05$) were seen in fifth graders versus first graders.

***Cell viabilities.*** There was a significant difference between nasal cell viabilities in control versus exposed children in first, second, and fifth grades ($p < 0.05$), but there were no differences between controls and third and fourth graders. The viabilities were 76.2 ± 6.2, 56.3 ± 12, 61.7 ± 13, 64.6 ± 9, 65.2 ± 12, and 59.1 ± 17 for controls and first through fifth grade, respectively (Figure 4).

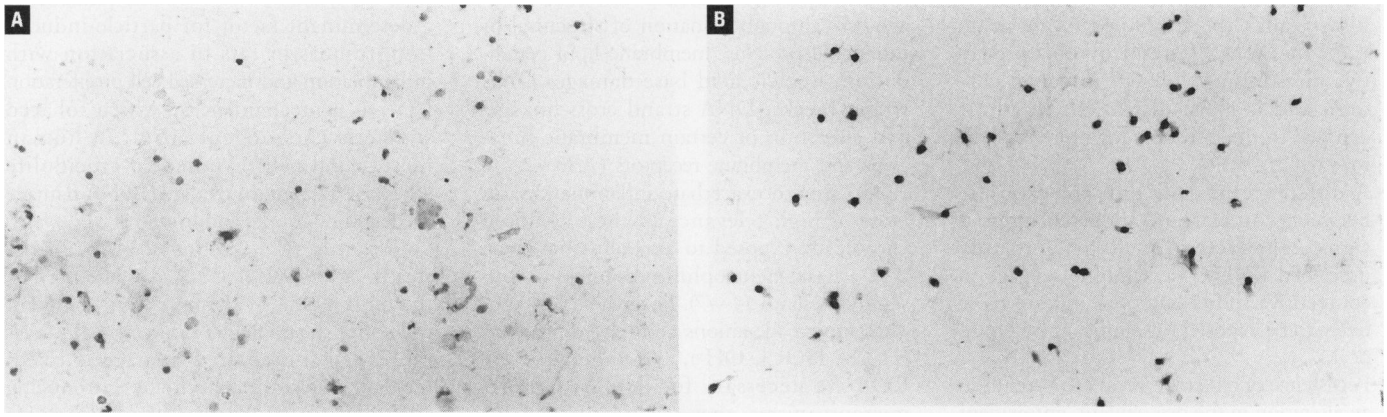


**Figure 1.** Immunohistochemical detection of 8-hydroxy-2′-deoxyguanosine using monoclonal antibody IF7 in nasal cells of (*A*) control and (*B*) exposed children. Darker nuclear staining is seen in the exposed child (*B*) versus the control child (*A*). Magnification × 200.

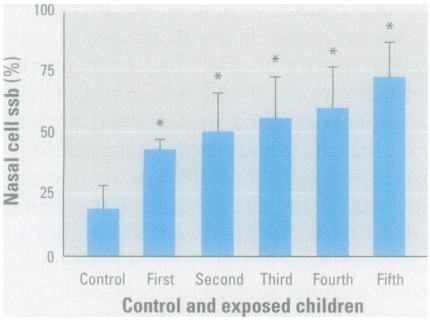


**Figure 2.** DNA single-strand breaks (ssb) in nasal cells from control versus exposed children grades one through five. An age-dependent increase in the percentage of cells with DNA tails > 10 μm is seen in southwest metropolitan Mexico City children.
*Significant difference between control and exposed groups ($p < 0.05$).

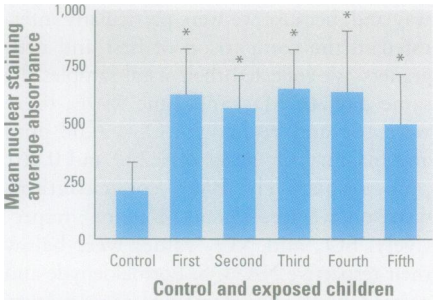


**Figure 3.** Mean nuclear staining (average absorbance) for 8-hydroxy-2′-deoxyguanosine in nasal cells using monoclonal antibody IF7; control versus exposed children grades one through five.
*Significant difference between control and exposed children ($p < 0.05$).

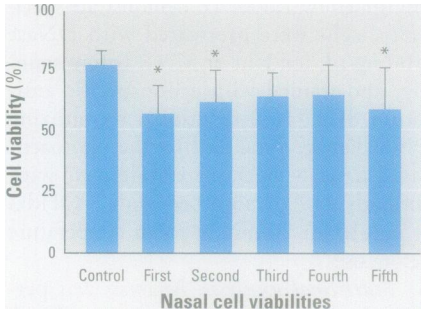


**Figure 4.** Nasal cell viabilities (expressed as a percent of total), as determined by the propidium iodide exclusion method; control versus exposed children grades one through five.
*Significant difference between control and exposed group ($p < 0.05$).

## Discussion

This study describes the simultaneous application of two methods of estimating different types of DNA damage to the study of nasal cells in children chronically exposed to severe air pollution: DNA ssb by the SCGE assay, and 8-OHdG (a product of base oxidation) detected by immunohistochemistry. Although we observed an age-dependent increase in the numbers of ssb, the 8-OHdG mean nuclear intensity—2.3 to 3-fold higher in exposed versus control children—remained the same for all five age groups studied. The age-dependent increase in the number of ssb in nasal cells has been previously described by this laboratory in a similar population of SWMMC elementary school children (*21*). These results could be related to several factors:

- The progressive diminution with age of patches of goblet cell hyperplasia, with decreased production of mucus and its antioxidant effects (*21,27*). Goblet cell metaplasia has been described in nasal transitional epithelium of monkeys exposed 8 hr/day for 6 days to relatively low ozone concentrations (0.15 ppm) and represents an adaptative protective mechanism against the continued ozone insult (*28*). In SWMMC residents this protective mechanism is present in young children and is markedly absent in adults exposed to the outdoors for more than 10 hr/day (*21,29,30*).
- A difference in daily outdoor exposure between younger and older children, a significant factor ($p < 0.001$) already described (*21*). Older children engage in competitive sports outdoors and are more likely to be exposed to significant pollutant peaks.
- Persistence of oxidative stress could result in an alteration in the pro-oxidant–antioxidant balance, a situation that might become more severe with time (*31*).
- The lack of differences could also be due to limitations of the technique—the immunohistochemical method of detection of 8-OHdG gives only relative levels of staining intensity, not quantitative data on damage levels. However, the combination of both techniques may be useful to detect oxidative stress in the nasal epithelia of these exposed children.

In Mexico City's environment, oxidative DNA damage is likely the result of the myriad of air pollutants present: $O_3$, $NO_2$, $NO_x$, $SO_2$, aldehydes, transition metals, automobile exhaust, and suspended particles that contain or act as reactive radicals (*27,32–36*). The respiratory epithelium is constantly exposed to ROS and RNS from exogenous (air pollutants, xenobiotics) as well as endogenous sources (phagocytic respiratory burst, mitochondrial respiration, cellular oxidases) (*7*). Excess production of ROS or RNS, their production in inappropriate relative amounts, or deficiencies in antioxidant defenses may result in pathological stress to the exposed cells and tissues (*37*). Our interest in free radicals in the Mexico City setting is related to the fact that they may contribute to human carcinogenesis by oxidative modification of DNA (*1,2,8,38–41*). ROS damage cells through oxidation of structural or functional proteins, membrane lipid peroxidation, nucleic acid base damage, DNA strand breaks, DNA strand cross-linkage, and alteration of certain membrane functions and membrane receptors (*7,38,42,43*). ROS can also exacerbate inflammation, an issue of high relevance in these children chronically exposed to air pollutants given that a nasal neutrophilic response is constantly present (*44–47*). Phagocytic cells produce superoxide anions and related products ($H_2O_2$, HOCl, $OH^{\cdot}$), and although these ROS are necessary for defense against microorganisms, they secondarily damage exposed host tissues (*7,48*). DNA damage caused by exposure to ROS such as hydroxyl radicals, singlet oxygen, superoxide radicals, and hydrogen peroxide is responsible for endogenous DNA alterations (*49*). Ames et al. (*2*) have proposed that oxidative DNA damage may be a significant causative factor for cancer; between $10^4$ and $10^5$ 8-OHdG residues are produced per cell each day in mammals. DNA replication past 8-OHdG promotes incorporation of adenine opposite the lesion and leads to G:C and T:A mutations. Oxidative DNA base damage (measured as 8-OHdG) has been used as a sensitive marker of oxidative stress in mouse lung carcinogenesis by Nagashima et al. (*50*) and Ichinose et al. (*51*). In the work of Nagashima et al. (*50*), instillation of diesel exhaust particles in mice tracheas *in vivo* produced a significant increase in 8-OHdG in the exposed lungs. The authors suggested that accumulation of 8-OHdG in lung DNA is a critical factor enhancing the rate of mutations in lung carcinogenesis. Increases in 8-OHdG have been reported in relation to the induction of both nonmelanoma and melanoma skin cancer by sunlight (*52*); rat liver carcinogenesis in postnatal life (*53*); and mouse hepatocarcinogenesis with exposures to tetrachloro-*p*-hydroquinone (*54*). In addition, the formation of 8-OHdG seems to be a determinant factor for particle-induced lung tumors in rats in association with inflammation and increased cell proliferation (*55*), as a mechanism by which tobacco smoke is carcinogenic (*56*), in human mesothelial cells exposed to crocidolite asbestos (*57*), and in oxidative DNA damage mediated by copper and iron (*58*).

According to Collins et al. (*42*), the steady-state level of DNA damage may be the most relevant parameter from the point of view of the etiology of cancer. As DNA is replicated, unrepaired damage is fixed; repair may also be inaccurate, introducing additional errors. Further, the capacity for repair of oxidative damage might vary between individuals [i.e., the balance between

several antioxidant enzymes (*38,39*)], and as a result of dietary factors (*59,60*).

DNA breaks may exert a long-range effect on chromatin conformation, which according to Cerutti (*38*) is incompatible with efficient transcription. Ozone genotoxicity has been demonstrated both *in vitro* and *in vivo* using DNA ssb as a biomarker (*61–63*). DNA ssb are increased in human lung fibroblasts and in a human bronchial epithelial cell line when incubated with arachidonic acid that has been degraded by ozone (*63*). *In vivo* controlled human exposures to 0.4 ppm ozone for 2 hr with moderate exercise induced DNA ssb in lung cells, mainly alveolar macrophages (*62*). Lee et al. (*62*) suggested that formation of DNA ssb may be an indicator of the tumorigenic potential of ozone. *In vivo* oxidative DNA damage is repaired continuously by a variety of enzymes; strand breaks are annealed and modified bases are excised as such or as nucleotides (*64*). Despite extensive repair, however, damaged nucleosides may accumulate if the sources of DNA damage are persistent. Antioxidant defense systems are often inadequate under a constant oxidant burden and damaged DNA leads to mutations and to an increasing risk for clonal expansion of cells transformed by oncogene activation and/or tumor-suppressor gene inactivation.

Oxidative DNA damage to the nasal respiratory epithelium is taking place in Mexico City residents (*21,29,30,44*) and multiple sources of ROS may be playing a crucial role in this persistent oxidative stress environment (*65,66*). Both nasal 8-OHdG and DNA ssb are significantly increased in exposed children as compared to controls and may be used as biomarkers for DNA damage in polluted environments. However, we agree with Spencer et al. (*67–69*) that care must be taken when using only one base product as a marker of oxidative DNA damage and that the interpretation of DNA ssb should take into account direct DNA damage versus oxidized base repair (*18,42*). Our main concern is that a persistent oxidative stress environment may ultimately result in the selective growth of preneoplastic nasal initiated cells (*40,66*) and that the potential for nasal neoplasms may increase as the children grow older. Enright et al. (*70*) suggest that the heterogeneity of damage to DNA with targeting of specific accessible sites is important in the development of malignancy, and that both the site specificity and the nature of the DNA lesions determine their carcinogenic potential. Additional studies of DNA damage and adduct formation (*71*) in the nasal passages of children living in Mexico City will be needed to make estimations of risk and impact of exposure to known and predicted carcinogens (*72*).

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